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Molecular motors: **Walking talking heads**

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Small tension signals that pass between the two linked heads of kinesin allow the motor protein to coordinate its walking action. Two new studies suggest that certain members of the two other major families of motor proteins, the myosins and dyneins, can do the same thing.

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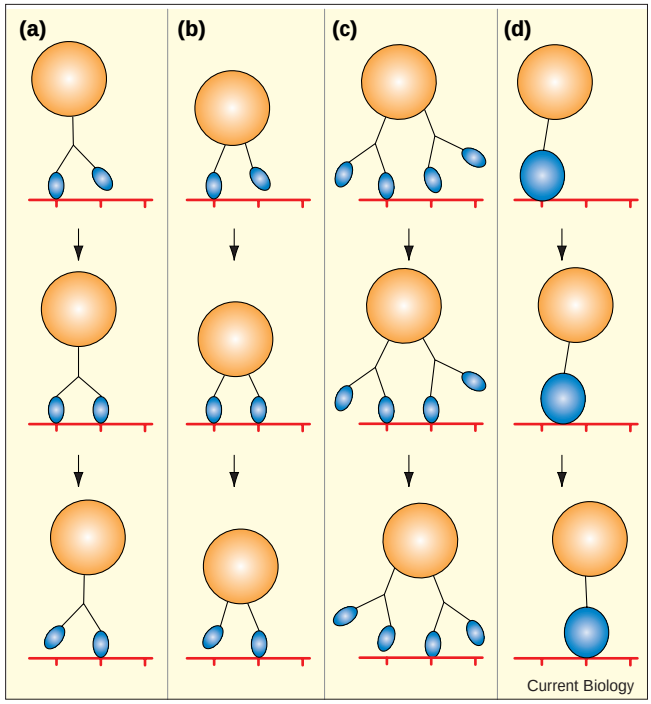
Next time you hurl something at an irksome collaborator, call to mind that ballistic success required the cooperation of around a trillion myosin molecules. Unlike those of their owners, the heads of the myosin molecules in our muscles are very good at seamless collaboration. Down among their teeming legions, any significant contretemps would tear your arm apart, as one set of myosins clung to the actin whilst others fought to dislodge them. In muscle, this alacritous teamwork originates in a property known as strain sensitivity, whereby each myosin head continuously monitors the forces generated by its neighbours, and responds by adjusting its own force-generating schedule to fit in. Strain sensitivity is a kind of intrinsic, rudimentary intelligence which is built into each myosin molecule, and which allows individual motors to be plugged together in series or in parallel, in a more or less freely scaleable fashion. The mutual awareness is so good that a 10^{12}-strong molecular collective of myosins is still 5–10-fold more efficient than your car engine.

There is now increasing evidence that even lower-level molecular conversations occur, between the linked heads of individual motor molecules. By coordinating their two heads, certain members of the kinesin family gain the ability to 'walk' along their microtubule track, with each head alternately gripping the microtubule track whilst the other changes binding site. This property, known as mechanical processivity, had been thought to be unique to the kinesins. But the results of two new studies [1,2] indicate that the other two great families of molecular motors, the myosins and the dyneins, also contain processive members.

Mehta *et al.* [1] report evidence that myosin V is a mechanically processive — 'walking' — motor. Myosin V has a provocative topology, each of its twin heads being joined to an exceptionally long neck region that is thought to act as a lever arm in the motor mechanism. Just looking at this molecule, the suspicion dawns that it has the molecular equivalent of seven league boots, and the race has been on to show that this is indeed so. Mehta *et al.* [1] found that actin filaments sliding over sparse surfaces of myosin V tend to pivot about their attachment point, reminiscent of microtubules sliding over brain kinesin [3,4] and suggesting that only a single myosin V molecule is driving motion.

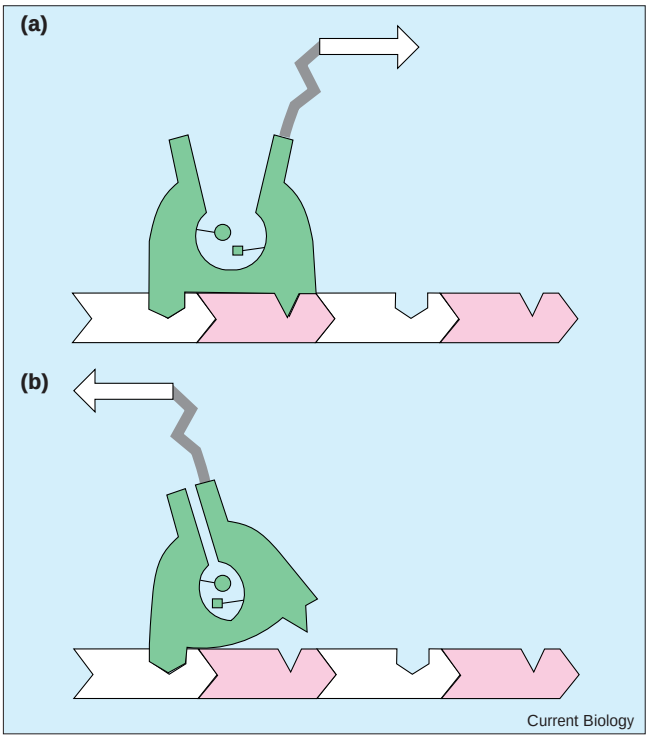
In search of numbers, Mehta *et al.* [1] then moved to a different geometry, previously used in pioneering experiments on the single (non-processive) interactions of muscle myosin with actin. Myosin V was attached at very low densities to polystyrene beads, and the beads attached to a coverslip. Biotinylated actin filaments were then caught between a pair of avidin-coated beads, and translated to the motor by gripping and steering the beads using twin optical traps.

Figure 1



Spot the processive motor. Processivity is defined as multiple mechanical steps per diffusional encounter with the microtubule. Two (or more) collaborating heads of the same motor molecule (**a**) are processive, as is one head able somehow to slip between sites (**d**), but collaborations between different molecules (**b**,**c**) are not *molecular* processivity. The key is the frame of reference: in (b) and (c) the bead is processive, but the individual molecules are not.

Figure 2



An imaginary mechanoenzyme, illustrating strain sensitivity. Pulling to the right, as in (a), has only modest effects. Pulling to the left, as in (b), closes the active site and reconfigures the catalytic residues (small circle and square), and also distorts the track binding interface, provoking detachment.

The traps were then parked (saving a small-amplitude oscillation that was used to monitor stiffness), and the positions of the beads at the ends of the actin filament tracked. In typical transients, the actin filaments were displaced in runs of three or four steps, with the size of the steps averaging 36 nm, exactly corresponding to the actin pseudorepeat (the axial distance between equivalent binding sites). Mehta *et al.* [1] conclude that myosin V is processive, with single mol-ecules able to step repeatedly against the retroactive force of the trap. A stall force of ~3 pN was sustained for up to several seconds before the motor detached.

Sakakibara *et al.* [2] report processivity in a very different motor, a single-headed dynein called inner arm dynein C, purified from *Chlamydomonas* flagellar axonemes. When coated at high density on to coverslips, this motor was found to drive microtubule sliding at over 5 μm per second. On more sparsely coated surfaces, the microtubules were driven more slowly — 0.7 μm per second — and methyl cellulose had to be added to the medium to stop the microtubules diffusing away from the surface. This sort of behaviour is characteristic of non-processive motors with a low 'duty ratio' — the proportion of a motor's ATPase cycle time which the molecule spends attached to the microtubule.

From their results, Sakakibara *et al.* [2] calculate a duty ratio for dynein C of 0.14. But the results of optical trapping assays revealed a surprise. In these assays, microtubules were attached to the coverslip and beads carrying single dynein C molecules gripped in a single beam optical trap and held close to the microtubule. Runs of multiple steps were observed. In weak traps, the bead–motor complexes translocated an average of 69 nm, corresponding to eight or nine steps, and reached a stall force of about 1.5 pN.

How can a single, low duty ratio motor head take runs of 8 nm steps against a restraining force? One important point is that the dynein head is large enough to bridge between adjacent binding sites on a microtubule protofilament, so that cooperating sites within the same head might be involved. An interesting possibility is that there are interacting, non-identical microtubule-binding sites within the dynein C head, one of which might be the stalk structure identified by Gee *et al.* [5] and visible in Sakaibara *et al.*'s [2] electron micrographs of inner arm dynein C. Another point is that surfaces of axonemal dyneins treated with an ADP.phosphate analogue can support diffusional motion of microtubules in sliding assays [6]. A weak binding (diffusional) mode may thus exist in the dynein cycle which could maintain contact of dynein C with microtubules between 'detachments', particularly when the motor is on a bead and the bead held close to the microtubule in an optical trap.

Sakibara *et al.* [2] point out that the maximum force that their motor can sustain is quite low, and that backsliding tends to occur once the motor is under tension. But weak as it is, this motor clearly can ratchet up multiple 8 nm steps against a restraining force. Is this achieved by coordinated binding and unbinding at two sites within the head, or do we, as Sakakibara and colleagues suggest, have to rethink?

Discussions amongst human collaborators about processivity have a tendency to lose focus, because whilst everyone agrees processivity is interesting, not everyone agrees exactly what it is. A major part of this is a frame-of-reference problem: two separate heads translocating an attached bead are not processive, whereas two heads of the same molecule doing the same thing are (see Figure 1). Rather than get into this, and associated wrangles about whether single molecule assays are really single molecules, consider the possibility that what is really important is strain sensitivity, the communication mechanism that coordinates processivity.

To ask how strain sensitivity works might seem premature, because exactly how a single head produces strain is still poorly understood. But we can make some general inferences about strain sensitivity without knowing the detailed

force-generating mechanisms — the structure and elasticity of the strain sensitive conformers — and can hope along the way to pick up clues about the force-generating mechanism. There are two main classes of explanation to think about, those in which the tension influences the rate constants of mechanical processes (for example, translating a head to its next site) and those which posit coupling between the tension and the probability of chemical steps. Self-consistent models can be built in the former class, but in fact we already now know that kinesin at least is tightly coupled [7–10], and that, as retrograde strain increases, so the chemical kinetic cycle slows down. The results reported by Mehta *et al.* [1] shows that, at higher forces, dwell times between myosin V steps increase, consistent with such coupling. Similar behaviour is apparent in the dynein C records. So we can rule out purely mechanical models and concentrate instead on the mechanisms by which tension influences the chemical kinetic rate constants of a motor, whilst bearing in mind that mechanical steps will be strain-sensitive also. Figure 2 shows an imaginary motor with both chemical steps and mechanical steps sensitive to the direction and magnitude of strain.

Strain sensitivity has been of major interest in the muscle field for some years, but molecular mechanisms have been difficult to pin down. With the advent of single molecule techniques it is now possible to ask which steps in the chemical cycle of ATP turnover are strain sensitive, and to measure the effects of strain — both magnitude and direction — on rate constants. This work is underway in several labs, and the answers should have general biological, and possibly nanotechnological, significance. Note, however, that communicating by straining your collaborators is of limited use for humans (I have tried it).

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